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13. ABSTRACT (Maximum 200 Words)

Prolonged exposure to estrogens is considered a major risk factor for development of breast cancer. When treated with estrogen for 28 weeks, ACI rats develop mammary cancers in over 90% of the population at risk. Genetic crosses between the susceptible ACI rat and resistant Copenhagen (COP) or Brown Norway (BN) rats identified a region on chromosome 5 (*Emca1*) that modified the development of estrogen-induced mammary cancer. To define the role of *Emca1* in the development of estrogen-induced mammary cancer, a congenic line has been developed (ACI.BN-*Emca1*) in which the resistant BN allele of *Emca1* has been introgressed onto an ACI background. Female ACI.BN-*Emca1* rats treated with estrogen for 28 weeks exhibit a significant decrease in the incidence of mammary cancer in the population at risk, a significant delay in the latency to the development of mammary cancer, and a significant decrease in the number of tumors per rat compared to ACI rats. These data suggest that *Emca1* is a strong modifier of estrogen-induced mammary cancer. Additional congenic lines, in which the *Emca1* locus has been divided into smaller regions, have been generated and will be used to further define the region(s) on chromosome 5 and to identify more precisely the gene(s) that modify estrogen-induced mammary cancer.

14. SUBJECT TERMS
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INTRODUCTION: Prolonged exposure to estrogens is considered a major risk factor for development of breast cancer. When treated with the naturally occurring estrogen, 17β-estradiol (E2), ACI rats develop mammary cancers in over 90% of the population at risk. Genetic crosses between the susceptible ACI rat and resistant Copenhagen (COP) or Brown Norway (BN) rats identified a region on chromosome 5 (*Emca1*) that modified the development of estrogen-induced mammary cancer. The purpose of this research is to determine the role of *Emca1* in the development of mammary cancer. To define the role of *Emca1* in the development of mammary cancer, a congenic line has been developed (ACI.BN-*Emca1*) in which the resistant BN allele of *Emca1* has been introgressed onto an ACI background.

BODY: The following accomplishments are documented according to the approved statement of work:

Task 1: **Evaluate the impact of *Emca1* on the development of estrogen-induced mammary cancer.**

- **Experimental design**: Female ACI, BN and ACI.BN-*Emca1* rats were treated with E2 beginning at nine weeks of age. Following five weeks of treatment, animals were examined twice weekly for the presence of palpable mammary tumors. Mammary tissues were collected following 12 or 28 weeks of E2 treatment.
- Female ACI.BN-*Emca1* rats were treated with E2 for 12 (7 rats) or 28 (44 rats) weeks.
- Data on ACI rats from these (14) and previous studies (62) were combined for statistical analysis.
- **Results**: Three indicators of reduced susceptibility to E2-induced mammary cancer were documented.
    - ACI.BN-*Emca1* females exhibited a significantly lower incidence ($P \leq 0.01$) of mammary cancer in the population at risk (66%) than the parental ACI rats (90%; Appendix I Figure 1).
    - ACI.BN-*Emca1*females developed significantly fewer ($p<0.05$) tumors per rat (1.98 ± 0.2) compared to parental ACI rats (4.96 ± 0.06; Appendix I Figure 2).
    - Latency to the development of mammary tumors was significantly prolonged ($p<0.001$) in the 29 ACI.BN-*Emca1* rats (171.5 ± 3.9 days) that developed mammary tumors compared to the parental ACI rats (136.0 ± 3.5 days; Appendix I Figure 3).
- **Status**: Study of the impact of *Emca1* on the development of E2-indcued mammary cancer completed. Analysis of mammary tissues from the 12 week study, including cell proliferation, lobuloalveolar hyperplasia and lobular density, and focal regions of atypical hyperplasia, is currently underway.

Task 2: Establish more precisely the location of the genes that confer and/or modify susceptibility to estrogen-induced mammary cancer.

- **Experimental Design**: Additional genotyping of large populations of F2 and backcross progeny from ACIxCOP and ACIxBN reciprocal crosses using polymorphic markers.
- **Results**: The *Emca1* locus could not be established more precisely.
    - Linkage analysis of two backcross populations (67 and 71 rats) from the BNxACI cross, using 16 polymorphic markers spanning *Emca1*, identified no correlation between genotype and any mammary phenotype.
    - Additional analysis of the ACIxCOP backcross populations was not completed

• Effort was directed toward further defining the *Emca1* locus in a population of 255 phenotypically defined (BNxACI)F_2 rats.

•Additional markers changed the shape of the tracing (Appendix I Figure 4), the region of interest on chromosome 5 remained the same.

• Statistical analysis indicated two models of inheritance at different regions within *Emca1*, suggesting the presence of at least two modifiers of estrogen-induced mammary cancer within *Emca1*. One modifier, between markers *D5Rat190* and *D5Rat98*, appeared to confer susceptibility to E2-induced mammary cancer in a recessive or incompletely dominant manner. A second modifier, between markers *D5Rat16* and *D5Rat33*, appeared to confer susceptibility to E2-induced mammary cancer in a dominant manner. In a population of 90 (ACIxBN)F_2 phenotypically females, a third region, between markers *D5Rat26* and *D5Rat95*, was identified that appeared to confer susceptibility to E2-induced mammary cancer in a recessive or incompletely dominant manner.

• Searching the *Ensembl* rat genome data base identified 154 known genes within this region. Of particular interest is the *cdkn2a* gene which encodes the p16 protein and is located between the two peak markers, *D5Rat153* and *D5Rat154*.

• **Status**: Task completed.

Task 3: Characterize additional congenic lines carrying specific intervals of *Emca1* to determine the effect of genotype on susceptibility to estrogen-induced mammary cancer.

• **Experimental Design**: Generate additional congenic lines based on information from Task 2.

• **Results**:

•Three additional congenic lines (*Emca1a*, *Emca1b*, and *Emca1c*) have been developed (Appendix I Figure 4). ACI.BN-*Emca1a* is bounded by markers *D5Rat190* and *D5Rat98*. ACI.BN-*Emca1b* is bounded by markers *D5Rat16* and *D5Rat33*. ACI.BN-*Emca1c* is bounded by markers *D5Rat95* and *D5Rat205*.

•Because the *cdkn2a* gene was located within the primary peak identified in the (BNxACI)F_2 population, a congenic line in which a 3.6 cM region of chromosome 5 containing the BN *cdnk2a* gene has been introgressed onto the ACI background, is also under development (ACI.BN-*Emca1-p16*).

• **Status**:

• Propagation of the ACI.BN-*Emca1a*, ACI.BN-*Emca1b*, and ACI.BN-*Emca1c* congenic lines is underway. Female rats will be treated with E2 when they reach nine weeks of age.

• One male and two females that are heterozygous for the *Emca1-p16* region are scheduled to be mated 7/14/04 to produce homozygous founders for the line.

REPORTABLE OUTCOMES:

Schaffer, B.S., McLaughlin, M.T., Tochacek, M., Pennington, K.L., Meza, J.L. and Shull, J.D. Cold Spring Harbor Rat Genomics & Models, Dec. 2003 Physical confirmation of *Emca3*, a locus that modifies estrogen-induced tumor multiplicity, in the ACI.BN-*Emca3* congenic rat strain. (Oral).

Gould, K.A., Tochacek, M., Schaffer, B.S., Reindl, T.M., Murrin, C.R., Lachel, C.M., VanderWoude, E.A., Pennington, K.L., Flood, L.A., Bynote, K.K., Meza, J.L., Newton, M.A. and Shull, J.D. 2004 Genetic Determination of Susceptibility to Estrogen-Induced Mammary Cancer in the ACI Rat: Mapping of *Emca1* and *Emca2* to Chromosomes 5 and 18 (manuscript submitted).

Strecker, T.E., Spady, T.J., Kaufman, A.E., Shen, F., McLaughlin, M.T., Pennington, K.L., Meza, J.L., Schaffer, B.S., Gould, K.A., and Shull, J.D. 2004 Genetic Bases of Estrogen-Induced Pituitary Tumorigenesis: Identification of Genetic Loci Determining Estrogen-Induced Pituitary Growth in Reciprocal Crosses between the ACI and Copenhagen Rat Strains (manuscript in press).

Schaffer, B.S., McLaughlin, M.T., Tochacek, M., Pennington, K.L., Meza, J.L. and Shull, J.D. 2004 Confirmation of *Emca1*, a locus that modifies development of estrogen-induced mammary tumors, in the ACI.BN-*Emca1* congenic rat strain. XVth International Workshop on Genetic Systems in the Rat (abstract accepted).

Schaffer, B.S., McLaughlin, M.T., Tochacek, M., Pennington, K.L., Meza, J.L., McComb, R.D. and Shull, J.D. 2004 Characterization of estrogen-induced mammary cancer in the ACI.BN-*Emca1* congenic rat: evidence that *Emca1* inhibits mammary carcinoma. San Antonio Breast Cancer Symposium (abstract submitted).

CONCLUSTIONS:

The ACI.BN-*Emca1* congenic line exhibited delayed latency to the development of E2-induced mammary cancer, decreased incidence within the population at risk, and decreased tumors per rat when compared with the susceptible ACI parental rats. Determining the susceptibility of the additional congenic lines developed in Task 3 will further refine the *Emca1*interval and aid in identifying the genes within *Emca1* that modify susceptibility to E2-induced mammary cancer.

APPENDIX I

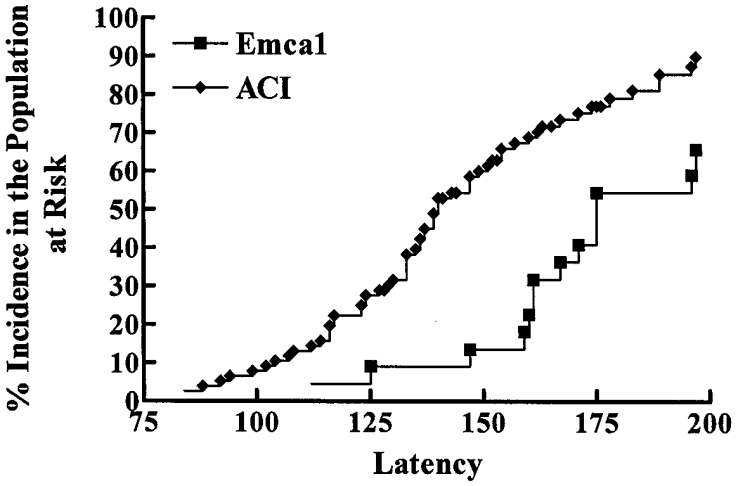

Figure 1. Incidence of mammary cancer in ACI.BN-*Emca1* rats. ACI (76) or ACI.BN-*Emca1*(44) rats were treated with E2 beginning at nine weeks of age and monitored twice weekly for the presence of mammary tumors. Each point represents the time at which an animal in the population at risk first exhibited a palpable mammary

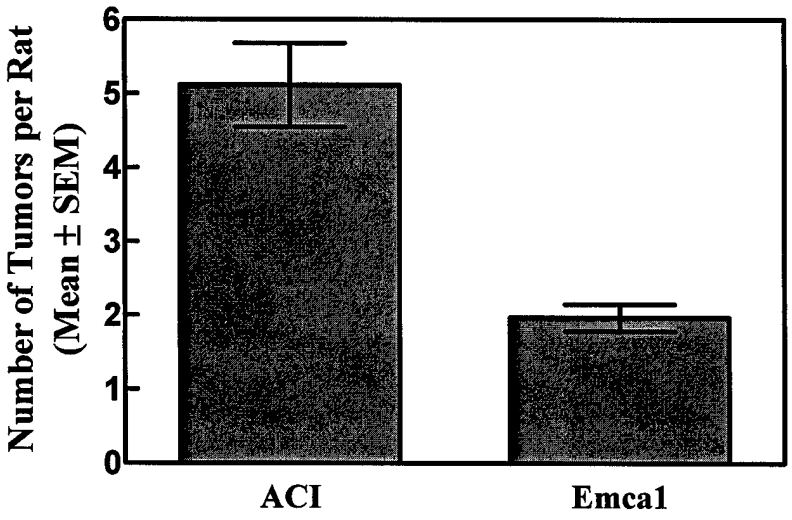

Figure 2. Tumor burden in ACI.BN-*Emca1* rats. ACI (62) and ACI.BN-*Emca1* (29) rats were sacrificed following 28 weeks of estrogen treatment and the number of tumors counted. Bars represent mean ± SEM.

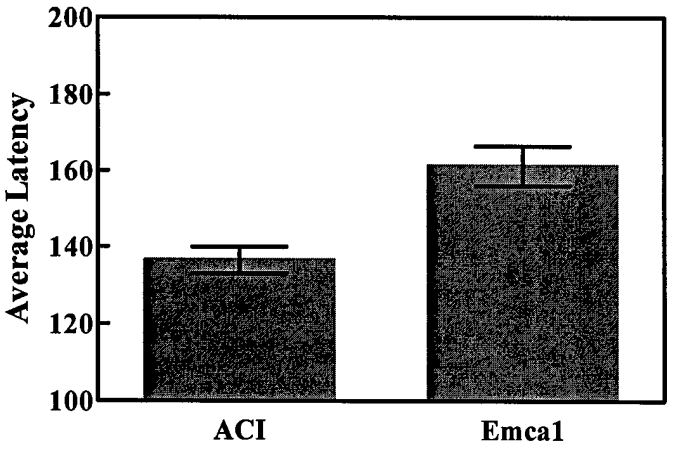

Figure 3. Latency to the development of mammary tumors in ACI.BN-*Emca1* rats. ACI (62) or ACI.BN-*Emca1* (29) female rats were treated with estrogen for 28 weeks and latency to the development of mammary tumors monitored. Bars represent mean ± SEM.

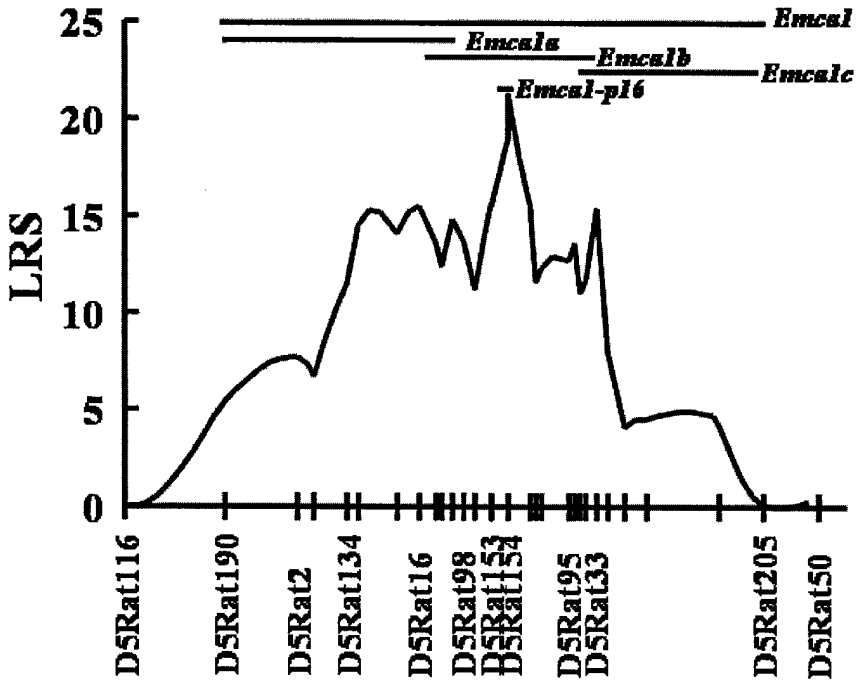

Figure 4. Development of *Emca1* congenic lines. The *Emca1* locus has been divided and multiple congenic lines developed to better define the region of interest on chromosome 5. Lines represent the region on chromosome 5 encompassed by the congenic lines.